[1-Deaminopenicillamine,4-valine]-8-D-arginine-vasopressin, a Highly Potent Inhibitor of the Vasopressor Response to Arginine-vasopressin[1]

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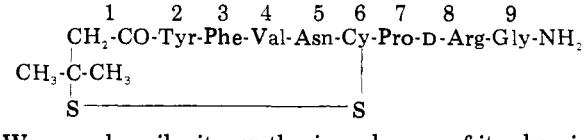
In attempting to design an antagonist of the antidiuretic response to arginine-vasopressin (AVP) [1-deamino-penicillamine,4-valine,8-D-arginine]vasopressin (dPVDAVP) was synthesized by the solid-phase method and assayed for antidiuretic, vasopressor, and oxytocic activities. dPVDAVP has an antidiuretic potency of 123 ± 22 units/mg, one-tenth that of its parent [deamino,4-valine,8-D-arginine]vasopressin (dVDAVP). Like dVDAVP its antidiuretic effect in conscious diabetes insipidus rats is greatly prolonged when compared to AVP. dPVDAVP causes a prolonged inhibition of vasopressor responses to AVP but not to norepinephrine or angiotensin II. It has an antivasopressor pA_2 value of 7.82 ± 0.05 when tested against AVP. Thus the penicillamine substitution at position 1 in dVDAVP increased its antivasopressor activity sixfold (dVDAVP has a pA_2 value of 7.03 ± 0.11). dPVDAVP is thus the most potent vasopressor antagonist yet reported. dPVDAVP was also found to be a potent inhibitor of the in vitro oxytocic response to oxytocin (pA_2 value = 7.23 ± 0.04). dPVDAVP with its potent and specific ability to antagonize the vasopressor effects of AVP should be a useful pharmacological tool with which to explore the possible participation of AVP's potent vasoconstrictor properties in cardiovascular regulation in physiological and pathological states.

An extensive study of factors which enhance the antidiuretic activity, specificity, and duration of action of arginine-vasopressin (AVP) culminated in the synthesis of the extraordinarily potent and specific antidiuretic agent [1-deamino,4-valine,8-D-arginine]vasopressin (dVDAVP).[2] This analogue shows a fourfold enhancement of antidiuretic activity over that of AVP, its antidiuretic action is greatly prolonged, and it has very low oxytocic potency and undetectable vasopressor activity.[2-4] In high doses dVDAVP causes a long-lasting inhibition of the vasopressor response to AVP.[3]

The incorporation of a residue of a 3-mercapto-3,3-dialkylpropanoic acid into position 1 of oxytocin,[5] deamino-oxytocin,[5-7] and deamino-lysine-vasopressin[8] has been found to endow the resulting peptides with a variety of antihormonal properties: inhibition of the oxytocic and avian vasodepressor responses to oxytocin and of the vasopressor response to lysine-vasopressin (LVP). The analogues tested have, however, no evident effect on the antidiuretic response to LVP.[9,10]

In searching for clues to the design of an antagonist of the antidiuretic response to AVP prior to the publication of the properties of [1-β-mercapto-β,β-diethylpropionic acid]-8-lysine-vasopressin ([1-β-Mpa(β-Et_2)]LVP),[8] we were intrigued by the possibility that incorporation of a β,β-dialkyl type substitution at position 1 of dVDAVP might convert this potent antidiuretic agonist into an antagonist of the antidiuretic response. Thus [1-deaminopenicillamine,Val^4,D-Arg^8]vasopressin (dPVDAVP) was designed according to this rationale. It has the following structure.



We now describe its synthesis and some of its chemical, physical, and pharmacological properties. For the synthesis of dPVDAVP the protected acyloctapeptide, 3-(*S*-benzylmercapto)-3,3-dimethylpropanoyl-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tox)-Gly-NH_2, was synthesized by the solid-phase method[11,12] using previously described procedures.[13,14] Deblocking of this intermediate was effected with Na in liquid NH_3[15] as previously described[13,14] and the resulting disulfydryl compound was subjected to oxidative cyclization with $K_3[Fe(CN)_6]$.[14,16] dPVDAVP was purified by gel filtration on Sephadex G-15.[17]

Bioassay Methods. Agonistic properties of dPVDAVP were assayed by previously published methods.[3,18,19] Antagonistic activities were tested by methods based on those introduced by Schild[20] and expressed by pA_2 values. The pA_2 represents the negative logarithm to the base 10 of the average molar concentration (M) of an antagonist which will reduce the specific biological response to $2X$ units of an agonist to the level of response to X units of the agonist. Antioxytocic activity was tested on the isolated rat uterus suspended in a magnesium-free van Dyke–Hastings solution.[21] The antagonist was added to the bath immediately prior to injection of the agonist which was the USP Posterior Pituitary Reference Standard. Antivasopressor pA_2 values were estimated by iv injection into urethane-anesthetized, phenoxybenzamine-treated rats, as in the standard rat vasopressor assay.[19] The antagonist was injected iv immediately before the injection of standard containing AVP. These antivasopressor pA_2 estimates must be considered as rough approximations since it is impossible to control the molarity of the peptide at the in vivo receptor site. One must assume uniform mixing and distribution in a fixed volume and rapid equilibration of peptide concentrations between this volume and the "receptor compartment". Dyckes et al.[10] assumed that antagonistic peptides were uniformly distributed in the blood volume. They assumed this to be 67 mL/kg in rats. This introduces possible error since peptides probably do not enter the red cell volume and the rat blood volume is usually less than 67 mL/kg. We have, however, followed the convention of Dyckes et al.[10] and considered 67 mL/kg to be the volume of distribution of antagonist so that we can compare our pA_2 estimates with theirs.

Results and Discussion

Preliminary experiments showed that dPVDAVP caused a prolonged inhibition of vasopressor responses to AVP but not to norepinephrine or angiotensin II. A single iv injection of 10 μg/kg of dPVDAVP antagonized vasopressor responses to AVP in doses of 10–40 mU/kg (about 25–100 ng/kg) for an average period of 2.5 h or more. In this respect it resembles dVDAVP which exerts a similarly prolonged but weaker antagonistic action.[3] The antidi-

Table I. Some Pharmacological Properties of dPVDAVP, dVDAVP, and [1-β-Mpa(β-Et_2)]-LVP

Peptide	Oxytocic act., U/mg	Antidiuretic act., U/mg	Antivasopressor pA_2	Antioxytocic pA_2
dPVDAVP	Anti	123 ± 22	7.82 ± 0.05	7.23 ± 0.04
dVDAVP	8	1230 ± 170	7.03 ± 0.11	
[1-β-Mpa(β-Et_2)]-LVP	Anti		7.15[a,b]	6.84[a]

[a] Values from Dyckes et al.[10] who calculated pA_2 values from the mean of the molar concentrations estimated for each assay group. We followed Schild's[20] original recommendation and calculated a pA_2 for each assay group and the figures presented are the means ± SE of these. If calculated by the method of Dyckes et al.,[10] antivasopressor pA_2 values would be 7.80 and 6.97 for dPVDAVP and dVDAVP, respectively, and the antioxytocic pA_2 for dPVDAVP would be 7.21. [b] Estimate based on assays using LVP as agonist.

uretic action of dPVDAVP injection sc into Brattleboro rats with hereditary hypothalamic diabetes insipidus[4] was also prolonged; 100 ng/kg inhibited diuresis for over 6 h. An equivalent duration of antidiuresis is seen after 10 ng/kg of dVDAVP, consistent with its tenfold greater antidiuretic activity (Table I); 100 μg/kg of AVP merely causes 1.7 h of antidiuresis although AVP has about three times the antidiuretic activity of dPVDAVP. The long duration of its antivasopressor and antidiuretic actions suggests that dPVDAVP, like dAVP,[22] is eliminated from the circulation much more slowly than is AVP.

We were naturally disappointed that penicillamine substitution in a highly active and specific antidiuretic peptide, dVDAVP, had not yielded an antidiuretic antagonist but had merely reduced antidiuretic agonistic activity by about 90%. Penicillamine substitution did, however, enhance the antivasopressor activity of dVDAVP about sixfold (Table I). The iv dose of dPVDAVP represented by its pA_2 value would be 1.1 μg/kg and this partially blocked responses to AVP challenge doses of 10 and 20 mU/kg (25 and 50 ng/kg).

It is impossible to make precise quantitative comparisons of the antivasopressor activity of dPVDAVP with those of other reported antivasopressor peptides[10,23,24] since these activities have been estimated by differing methods. Bisset et al.,[23] for example, reported that doses of 60 μg or more of [1-*N*-carbamoylhemicystine,2-*O*-methyltyrosine]oxytocin were needed to appreciably reduce vasopressor responses to 20 ng of AVP in pithed rats. This analogue would appear clearly weaker than dPVDAVP which inhibits in doses of about 0.25 ng per rat. Cort et al.[24] report that the 4-isoleucine and 4-leucine analogues of dVDAVP were 2 and 2.5 times more potent antagonists than dVDAVP. Since we find that dVDAVP has a pA_2 of 7.03 this would suggest that they may have pA_2 values of about 7.3 and 7.4 although these were not estimated directly by Cort et al.[24] Dyckes et al.[10] report that [1-(β-mercapto-β,β-diethylpropionic acid)]lysine-vasopressin has a pA_2 value of 7.15 (Table I). They used LVP as an agonist but the method was otherwise comparable to the method we used. LVP is a somewhat weaker vasopressor agonist than AVP. If we assume that this did not appreciably affect the pA_2 estimates, it appears that dPVDAVP, with a pA_2 of 7.82, is at least six times as potent. From their published variances of the effective molarities of their analogue, it appears that the difference between it and dPVDAVP is highly significant statistically.

Thus dPVDAVP appears to be the most potent antagonist of the vasopressor effects of AVP yet reported. Such an active and specific antagonist of the vasopressor effects of AVP should be a useful pharmacological tool with which to explore the possible participation of AVP's potent vasoconstrictor properties in cardiovascular regulation in physiological and pathological states.

Experimental Section

The Boc-Gly resin was supplied by Schwarz Bioresearch and *tert*-butyloxycarbonylamino acids by Schwarz Bioresearch, Bachem, or Biosynthetika. 3-(*S*-Benzylmercapto)-3,3-dimethylpropanoic acid was prepared according to the literature.[5] Reagents and solvents were analytical grade. The chemical analysis was performed by Galbraith Laboratories, Inc., Knoxville, Tenn. The analytical results for the elements indicated by their symbols were within ±0.4% of theoretical values. For amino acid analysis[25] samples were hydrolyzed with constant boiling HCl in evacuated, sealed ampules, for 20 h at 110 °C. The analyses were performed with a Beckman/Spinco amino acid analyzer, Model 121C; molar ratios are referred to Gly = 1.00. Optical rotations were measured with a Bellingham Stanley Ltd. Model A polarimeter, Type P1. R_f values are for chromatography on silica gel (Brinkman Silplate, 0.25 mm) in the following solvent systems: A, butan-1-ol-acetic acid-water (4:1:5 v/v, upper phase); B, butan-1-ol-acetic acid-water-pyridine (15:3:3:10, v/v). Loads of 25–50 μg were applied and chromatograms were of minimum length, 10 cm. The chloroplatinate reagent, chlorine–potassium iodide–tolidine, and iodine vapor were used for detection.

3-(*S*-Benzylmercapto)-3,3-dimethylpropanoyl-Tyr(Bzl)-Phe-Val-Asn-Cys(Bzl)-Pro-D-Arg(Tos)-Gly-NH_2. Boc-Gly resin (6 g, 1.5 mmol of Gly) was carried through the four cycles of deprotection, neutralization, and coupling to yield the protected C-terminal pentapeptide resin.[13,14] A portion of this resin[26] (0.82 mmol based on chloride values) was subjected to the four further cycles required to afford the desired acyloctapeptide resin (3.83 g, 53% based on the glycine incorporation in the resin). The peptidyl resin (3.77 g) was ammonolyzed[13] and the product extracted with warm (60 °C) dimethylformamide (DMF). The solvent was evaporated in vacuo and the residue triturated with 95% ethanol and methanol. The crude product (466 mg) was reprecipitated from glacial acetic acid with 95% ethanol, and washed with ether, to yield the pure acyloctapeptide amide as a white powder, wt 363 mg. This represents an overall yield of 31% based on the initial glycine incorporation on the resin: mp 224–226 °C; $[\alpha]^{24}_D$ −26° (*c* 1, DMF); R_f (A) 0.65. Anal. ($C_{76}H_{95}N_{13}O_{13}S_3$) C, H, N.

[1-Deamino-penicillamine]-4-valine-8-D-arginine-vasopressin (dPVDAVP). A solution of the protected acyloctapeptide amide (150 mg, 0.1 mmol) in sodium dried, and redistilled, ammonia (ca. 300 mL) was treated at the boiling point and with stirring with sodium[15] by the intermittent introduction of a stick of metal contained in a small bore glass tube[14,16] until a "blue" color persisted in the solution for 30 s. Dry glacial acetic acid (ca. 0.5 mL) was added to discharge the color and the solution then evaporated. The residue was dissolved in aqueous acetic acid (0.2 M, 600 mL) and this solution treated with 2 M ammonium hydroxide solution to give a solution of pH 6–7. An excess of a solution of potassium ferricyanide (0.11 M, 19 mL) was added gradually and with vigorous stirring over 25 min. The yellow solution was stirred with anion-exchange resin (Bio-Rad AG3 X4, chloride form, ca. 10 g damp weight). The resin was washed with aqueous acetic acid (0.2 M, 300 mL) and the combined filtrate and washings were lyophilized. The residue was dissolved in aqueous acetic acid (50%, 10 mL) and this solution washed onto Sephadex G-15 (column 110 × 2.7 cm, prepared in 50% aqueous acetic acid).[17] The column was eluted with 50% aqueous acetic acid, flow rate ca. 4 mL h^{-1}, and the eluate was monitored for absorbance at 280 nm and fractionated. The fractions comprising the major peak were pooled and lyophilized, and the residue was subjected further to gel filtration of Sephadex G-15 (column 100 × 1.5 cm) with aqueous acetic acid (0.2 M, flow rate ca. 6 mL h^{-1}) as the eluting solvent.[17] Fractions (2.5 mL) were collected and

lyophilization of those comprising the single peak, detected by monitoring the absorbance of the eluate at 280 nm, yielded the ninhydrin-negative product (70 mg, 65%, yield overall, 20%): R_f (A) 0.26, R_f (B) 0.37; $[\alpha]^{22}_D$ −12° (c 0.5, 1 M acetic acid); homogeneous to electrophoresis in two pyridine acetate buffers (pH 3.5 and 6.5) and in aqueous acetic acid (30%). Amino acid analysis[21] gave the following molar ratios: Gly, 1.00; Arg, 0.99; Pro, 1.08; Asp, 1.02; Val, 0.99; Phe, 1.02; Tyr, 0.99; Cys, 0.14; NH_3, 2.0. A sample hydrolyzed identically after performic acid oxidation by the method of Moore[27] had a cysteic acid to Gly ratio of 1.02:1.00.

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Book Reviews

Receptors and Mechanism of Action of Steroid Hormones. Part I. Edited by George R. Pasqualini. Modern Pharmacology–Toxicology. Volume 8. Marcel Dekker, New York, N.Y., and Basel, Switzerland. 1976. xi + 309 pp. 16 × 23.5 cm. $29.75.

The discovery of proteins which can selectively bind the steroid hormones in target cells and that exhibit various properties likely attributable to the function of cellular receptors is a most significant development in this area. This monograph (part I of two parts) addresses this subject in detail describing recent (up to 1975) research on steroid hormones at the molecular level. Abridged chapter titles (authors) are 1, General Aspects of Receptor Interactions (Munck); 2, Autoradiographic Localization in Target and Nontarget Tissues (Stumpf, Sai); 3, Estrogen Receptor Purification by Affinity Chromatography (Sica, Cuatracasas, Nola, Parikh, Puca); 4, Estrogen in Normal Human Endometrium (Gurpide, Tseng); 5, Androgen Receptors and Mechanism of Action (Liao); 6, Control of Tumor Growth (King, Cambray, Jagus-Smith, Robinson, Smith); 7, Progesterone Mechanism of Action (Spelsberg, Taft). Chapters 8–12 are found in Part II and deal with Glucocorticoid Receptors (Munck, Leung); Mineralocorticoid Receptors (Pasqualini, Sumida); Aldosterone Mechanism of Action (Crabbé); Breast Cancer (Jensen, DeSombre); Receptors in Brain, Hypothalmus and Hypophysis (Kato). It is unfortunate that all chapters are not found under one cover; the cummulative index is found in Part II. For Part I each chapter is individually referenced mainly through 1974 with some 1975 citations.

Chapter 1 sets the stage for this work by including discussions of historical aspects, terminology, definition of receptors, and an overview of theoretical considerations. This chapter is well written, informative, and very interesting to read. The remaining chapters found in Part I are also well done; in the remaining chapters the title subject is treated in considerably more detail. For example, chapter 2 contains discussions of autoradiographic methods used to search for and define steroid hormone target sites and the results of these experiments. In chapter 3, details of affinity chromatographic methods, estrogen and polymer properties, and assay methods for purified receptors and their characterization are considered. Chapter 4 contains a summary of the studies in